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Natural Killer Cells Do Not Play a Dominant Role in CD4$^+$ Subset Differentiation in *Candida albicans*-Infected Mice

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The effects of in vivo administration of monoclonal antibodies against NK-1.1-bearing cells on the early production of gamma interferon (IFN-γ) in vitro and development of Th1-associated immunity were studied in mice infected with a live vaccine strain of *Candida albicans*. At 1 and 4 days postinfection, natural killer (NK) cell-enriched fractions from the spleens of antibody-treated mice displayed a dramatic reduction in 5E6$^+$ lymphocytes and negligible anti-YAC-1 cytotoxic activity in vitro. Nevertheless, the frequency of IFN-γ-producing cells in those fractions was reduced by less than half, on average, by anti-NK-1.1 treatment in vivo. In addition, the antibody-treated and infected mice demonstrated unchanged T helper cell responses, as measured by yeast-specific footpad reactions, resistance to reinfection, occurrence of antibodies of different isotypes, and production in vitro of interleukin-2 (IL-2), IFN-γ, IL-4, and IL-10 by CD4$^+$ cells. Therefore, although NK cells may contribute to early IFN-γ production in *Candida*-vaccinated mice, these cells apparently do not play a dominant role in the qualitative development of yeast-specific T helper responses.

Systemic inoculation of mice with the fungus *Candida albicans* can cause either a fatal disseminated disease or a self-limited infection depending on numerous factors, including genetic background of the mice (22) and nature of the yeast strain used for challenge (7, 8, 21). In both cases, a strong correlation is found between disease outcome and nature of the predominant T helper (Th) cell response, with healing infection involving a Th1-like pattern of reactivity and nonhealer mice developing a preponderance of Th2 responses (6, 24). However, alteration of the disease outcome and Th conversion are obtained in healer and nonhealer mice by changing their immune status at the time of infection (20, 23). We have recently shown that a single injection of an anti-gamma interferon (anti-IFN-γ) antibody into healer mice infected with a live vaccine strain (LVS) of *C. albicans* results in the detection of Th2 responses, alters the early expression of interleukin-4 (IL-4) and IFN-γ messages in CD4$^+$ cells, and prevents the onset of long-lived antifungal protection (20, 21).

Although these studies demonstrated that IFN-γ is crucial in regulating Th subset differentiation in *C. albicans*-infected mice, they did not identify the major cells responsible for the early production of IFN-γ, and both CD4$^+$ and CD4$^-$ CD8$^-$ cells were found to contribute to the effect (21). Because natural killer (NK) lymphocytes are known to be activated by *C. albicans* (9, 10, 29) and may secrete IFN-γ in response to microbial pathogens (11), they could play an important role in the early release of IFN-γ that leads to protective immunity.

The purpose of the present study was to examine whether NK cells are required for the production of IFN-γ that is essential for the development of Th1-associated protection in mice infected with a *C. albicans* LVS. By selective depletion through an anti-NK cell antibody, we obtained evidence that these cells did contribute to the early production of IFN-γ, yet their deficiency failed to negate the induction of protective immunity and did not modify the expression of the predominant (Th1) phenotype in healer mice.

MATERIALS AND METHODS

Mice. Inbred C57BL/6 and BALB/c mice were obtained from Charles River Breeding Laboratories, Calco, Milan, Italy. C57BL/6 mice of both sexes, ranging in age from 6 to 8 weeks, were used for the in vivo infection experiments.

Yeasts and infections. The origin and characteristics of the *C. albicans* highly virulent CA-6 strain and the LVS PCA-2 used in this study have already been described in detail (7, 8, 21). Yeast cells were grown to stationary phase at 28°C under slight agitation in low-glucose Winge medium composed of 0.2% (wt/vol) glucose and 0.3% (wt/vol) yeast extract (BBL Microbiology Systems, Cockeysville, Md.). After a 24-h culture, cells were harvested by low-speed centrifugation (1,000 × *g*), washed twice in saline, and diluted to the desired density to be injected intravenously into mice in a volume of 0.5 ml.

In vivo NK cell depletion. The hybridoma line PK136 producing immunoglobulin G2a (IgG2a) monoclonal antibody (MAb) to the NK-1.1 alloantigen (14) was obtained from the American Type Culture Collection (Rockville, Md.) and grown as ascites in pristane-primed, irradiated BALB/c mice. The ascitic fluid was passed over a desalting column (10DG; Bio-Rad Laboratories, Richmond, Calif.) prior to antibody purification by means of affinity chromatography with protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) (21). The protein concentration was determined by measuring the optical density at 280 nm. One day before infection, and again on days 1 and 3 of infection, mice were treated with 0.5 mg of purified MAb in 0.25 ml of phosphate-buffered saline intraperitoneally. Control mice received equivalent amounts of affinity-purified rat IgG (Zymed Laboratories, San Francisco, Calif.). To determine the effect of

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this procedure on NK activity, spleen cells from antibody-treated mice were collected and fractionated, and cell fractions were examined by flow cytometry or used in a 4-h ^{51}Cr-release assay against NK-sensitive YAC-1 targets by following standard procedures. To rule out the possibility that treatment with anti-NK-1.1 in vivo might be changing the cytotoxic potential of NK cells rather than depleting cytotoxic effectors, splenic populations from infected mice were exposed overnight to 1 to 10 μg of PK136 MAb per ml in vitro prior to being tested for lysis of YAC-1 targets, and this resulted in no detectable impairment of effector cell cytotoxic potential.

Enrichment for NK cells by density gradients. To enrich for NK cells in nylon wool-nonadherent splenic populations, discontinuous density gradients were prepared with Percoll (Pharmacia) as described previously (11, 17). Of the five fractions resulting from a discontinuous gradient of 70 to 30%, the low-density fraction, Percoll fraction 2, was used in this study as an NK cell-enriched population. In infected control animals, the percent input recovered in fraction 2 was approximately 25%, and these cells exhibited a high proportion (~35%) of large granular lymphocytes in stained cytospin preparations, which was concomitant with enrichment of anti-YAC-1 cytotoxic activity. After treatment with anti-NK-1.1 MAb, the level of recovery for fraction 2 was approximately 15%, but neither large granular lymphocytes nor appreciable cytotoxic activity was found in any Percoll fraction.

Cytofluorometry. The efficiency of NK cell depletion was confirmed by flow cytofluorometric analysis of Percoll fraction 2. Cells were incubated with fluorescein isothiocyanate-labeled mouse anti-mouse NK cell MAb 5E6 (Pharmingen, San Diego, Calif.) that reacts with cell surface molecules which are different from the conventional NK-1.1 antigens (27). After being washed, cells were analyzed with a FACScan cytofluorometer (Becton Dickinson, Sunnyvale, Calif.) as described previously (21). Data are percentages of positive cells after subtraction of background staining (i.e., with irrelevant fluoresceinated mouse MAb).

Spot ELISA. The procedure used to enumerate IFN-γ-producing cells in Percoll fractions was based on a spot enzyme-linked immunosorbent assay (ELISA), described by Dunn and North (11), using 96-well nitrocellulose membrane filtration plates (Millititre HA; Millipore Corp., Bedford, Mass.) precoated with rat anti-murine IFN-γ MAb R4-6A2 (23). Cells were incubated overnight with medium alone or plus heat-inactivated *C. albicans* or with 5 μg of phytohemagglutinin (PHA) per well. The secondary antibody was biotinylated MAb AN-18.17.24 (24), the enzyme was avidin-alkaline phosphatase conjugate (Vector Laboratories, Burlingame, Calif.), and the substrate was 5-bromo-4-chloro-3-indolylphosphate-*p*-toluidine salt (BRL Life Technologies, Gaithersburg, Md.). The results were expressed as the mean number of IFN-γ-producing cells per 10^5 cells, calculated by using replicates of serial twofold dilutions of cells pooled from three mice per group per experiment.

Selection of CD4$^+$ cells, production of culture supernatants containing cytokine activity, and cytokine assays. CD4$^+$ lymphocytes were positively selected from pools of spleen cells by means of a panning procedure using anti-murine CD4 MAb GK1.5 (7, 8, 24). Supernatants from mixed lymphocyte-*Candida* cultures were obtained as described previously (23, 24). IFN-γ, IL-2, IL-4, and IL-10 assays, together with the source and characteristics of the anticytokine antibody reagents, have been described previously in detail (22–24). Briefly, cell supernatants were tested for their concentration of IFN-γ by two-site ELISA using rat anti-murine IFN-γ MAb R4-6A2 as the primary antibody and biotinylated MAb AN-18.17.24 as the secondary antibody. IL-2 and IL-4 were measured in a colorimetric assay using the HT2 T-lymphocyte line that proliferates in response to either of these cytokines, and MAbs S4B6 (anti-IL-2) and 11B11 (anti-IL-4), at neutralizing concentrations, were used to establish monospecificity. The IL-10 assay was performed by two-site ELISA using anti-IL-10 MAb SXC-2, biotinylated MAb SXC-1, and peroxidase-labeled streptavidin. All cytokine titers were expressed as units per milliliter, calculated by reference to standard curves constructed with known amounts of recombinant cytokines (22).

Antibody assay. A micro-ELISA procedure was used to quantitate specific antibodies in the sera of yeast-infected mice (23, 24). The assay involved coating the microtiter plate wells with CA-6 antigen, adding appropriate dilutions of test antisera (from a pool of two to three mice per group), and a further reaction with alkaline phosphatase-conjugated rabbit anti-mouse IgG1, IgG2a, or IgG2b (Zymed Laboratories) or with biotinylated rat MAb anti-mouse IgE (Sanbio bv Biological Products, Uden, The Netherlands) and then with avidin-alkaline phosphatase conjugate. After addition of the substrate solution, the optical density of triplicate samples was read with an ELISA reader with a 405-nm filter.

DTH assay. A direct assay system for measuring the delayed-type hypersensitivity (DTH) response to cell surface antigens (8, 24), in which heat-inactivated CA-6 cells (2×10^6/0.04 ml of saline) were inoculated into the footpads of yeast-infected mice, was employed. The DTH reaction was recorded 24 h later by weighing the footpad as a measure of swelling, and results were expressed as the increase in footpad weight over that of the saline-injected counterpart. Data are the means ± the standard errors of eight mice per group.

RESULTS

Effect of NK-1.1$^+$ cell depletion on the course of primary candidiasis and resistance to reinfection. In a first series of experiments, C57BL/6 mice, which are known to be genetically resistant to candidiasis (4, 12), were treated with anti-NK-1.1 antibody starting 1 day before infection with 10^6 blastoconidia of the LVS PCA-2. Depletion of splenic NK cells was confirmed by cytofluorometric analysis of the 5E6$^+$ subset (Fig. 1A) in nylon wool-nonadherent cells in Percoll fraction 2 and by assessment of anti-YAC-1 cytotoxic potential in vitro (Fig. 1B). The antibody-treated and infected mice were monitored for mortality parameters, and after 2 weeks survivors were rechallenged with 10^6 cells of the highly virulent strain CA-6 (Table 1). It is apparent that the PCA-2-infected mice treated with control or anti-NK-1.1 antibodies resisted primary challenge and developed protective anticandidal immunity, despite the severe impairment in anti-YAC-1 cytotoxic activity resulting from NK cell deficiency. This suggested that the development of protective Th1 responses in PCA-2-infected mice might not be modified by anti-NK-1.1 treatment.

Development of *Candida*-specific footpad reaction. Prior studies have demonstrated a strong correlation between the development of DTH responses and the onset of protective anticandidal immunity in mice vaccinated with a LVS of *C. albicans* (7, 8, 24) or receiving anti-IL-4 therapy throughout the course of primary infection with CA-6 (23). To assess the pattern of DTH reactivity in NK-1.1$^+$ cell-depleted mice, groups of animals infected with 10^6 PCA-2 cells were in-

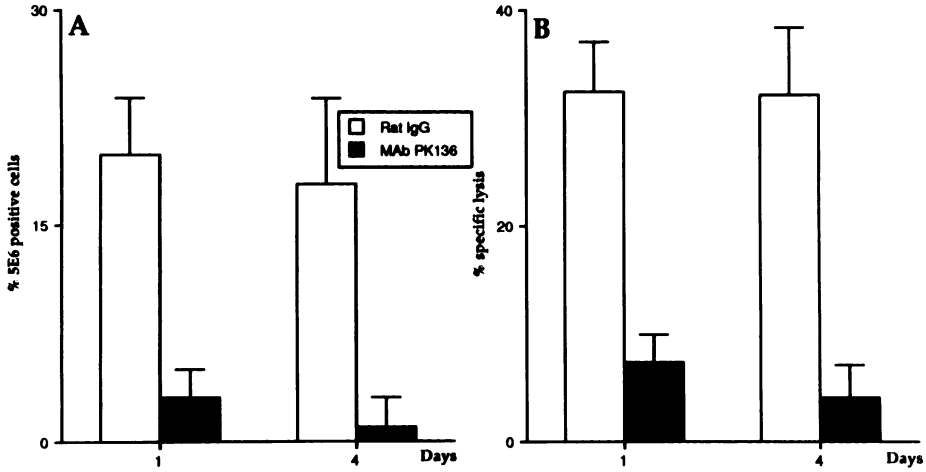

FIG. 1. Effect of in vivo administration of PK136 MAb on percentage of $5E6^+$ cells (A) and anti-YAC-1 cytotoxic activity (B) in Percoll-fractionated splenic populations from mice infected with *C. albicans*. Percoll-fractionated cells from mice infected with *C. albicans* for 1 or 4 days and treated once or thrice, respectively, with control or PK136 antibodies were subjected to cytofluorometric analysis or assayed for lysis of tumor target cells at an effector-to-target-cell ratio of 100:1. Data are the means ± the standard errors of three experiments, each involving pooled cells from three mice per group per experiment.

jected intrafootpad with heat-inactivated blastoconidia 1 or 2 weeks after infection. Twenty-four hours later, the increase in footpad weight was measured. Figure 2 shows that the development of a strong DTH reactivity was not affected by the ablation of $NK\text{-}1.1^+$ cells.

Effect of MAb treatment on B-cell isotype expression. Because protective anticandidal responses are often concomitant with high DTH expression and either low antibody production or antibodies mostly of the IgG2a isotype (7, 8, 23, 24), we measured the levels of yeast-specific antibodies in the sera of anti-NK-1.1-treated mice infected with PCA-2 cells. At 1 and 2 weeks after the injection of 10^5 blastoconidia, sera were collected and assayed for their contents of IgG2a and IgG2b, which are Th1-associated isotypes, or IgG1 and IgE, which are Th2-associated isotypes (16). Figure 3 shows that the ablation of $NK\text{-}1.1^+$ cells had no effect on regulation of B-cell isotype expression in vaccinated mice.

Effect of $NK\text{-}1.1^+$ cell depletion on cytokine production in vitro. The development of Th1 and Th2 responses is associated with the detection of different cytokine secretion profiles in vitro, which may account in vivo for most of the reciprocal cross-inhibition of Th1 and Th2 cells (16). To assess the pattern of cytokine release in vitro by purified $CD4^+$ lymphocytes from LVS-infected mice treated with control or PK136 antibodies, mixed lymphocyte-yeast cell cultures were established in the presence of accessory cells at 1 and 2 weeks after the injection of 10^5 PCA-2 cells. Appropriate controls included $CD4^+$ cells from intact donors and yeast-primed $CD4^+$ lymphocytes cultured with accessory cells but no fungal antigen. After 24 h of incubation, culture supernatants were assayed for their contents of IFN-γ, IL-2, IL-4, and IL-10. Figure 4 shows that the predominant Th1-like cytokine secretion pattern of LVS-infected mice was not modified by the depletion of NK-1.1-bearing cells.

Effect of $NK\text{-}1.1^+$ cell depletion on numbers of splenic IFN-γ-secreting cells. To directly investigate the early pattern of IFN-γ production in vitro and the effect of anti-NK-1.1 treatment, a spot ELISA was used to enumerate IFN-γ-secreting cells in NK cell-enriched splenic populations from mice treated with control or PK136 antibodies and infected

TABLE 1. Effect of anti-NK-1.1 treatment on development of anticandidal protective immunity

Treatment	Infection with[a]:			
	PCA-2		CA-6	
	MST	D/T	MST	D/T
Rat IgG	>60	0/8	>60	0/15
MAb PK136	>60	1/8	>60	0/15
No PCA-2			3	16/16

[a] Mice treated with control or anti-NK-1.1 antibodies on days −1, 1, and 3 were infected with 10^6 PCA-2 cells (on day 0) in three different experiments (eight animals per group per experiment). In two experiments, survivors of PCA-2 challenge (15 of 16 for both control and MAb treatment groups) together with intact controls (no PCA-2) were injected after 2 weeks with 10^6 yeast cells of the highly virulent strain CA-6. Median survival times (MST, in days) and the number of dead mice at 60 days over total number of animals tested (D/T) were recorded.

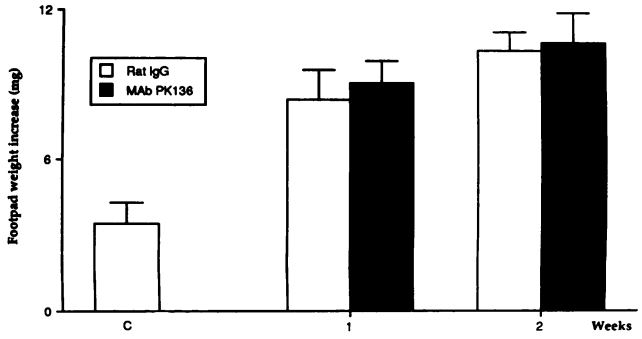

FIG. 2. DTH in *C. albicans*-infected mice receiving three injections of PK136 or control antibodies in one experiment of two. At 1 or 2 weeks after PCA-2 challenge, mice were injected intrafootpad with inactivated yeast cells 24 h before measurement of their footpad response. As a control, uninfected mice (C) were assayed. All values are significantly different ($P < 0.01$) from the value obtained in the controls, as determined by Student's *t* test.

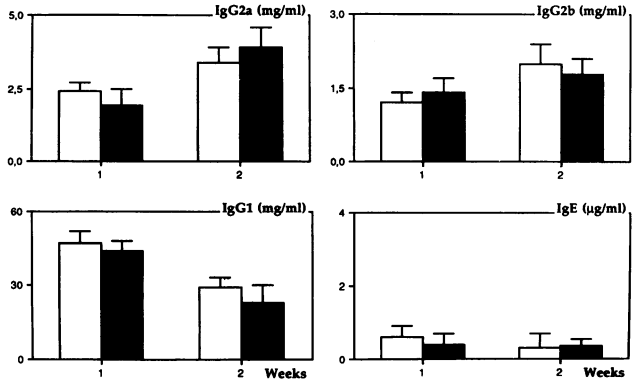

FIG. 3. Serum antibody levels in *C. albicans*-infected mice receiving three injections of PK136 (■) or control (□) antibodies. At 1 or 2 weeks after PCA-2 infection, sera were assayed for IgG2a, IgG2b, IgG1, and IgE titers. Values represent the means ± the standard errors of three experiments. Specific antibody levels in uninfected mice were negligible or undetectable.

for 1 or 4 days with PCA-2. Therefore, cells in Percoll fraction 2 were analyzed for frequency of IFN-γ-secreting cells under basal conditions or after in vitro stimulation with heat-inactivated *C. albicans* or PHA (Fig. 5). The results indicated that, early after LVS infection, the Percoll-fractionated population contained cells producing IFN-γ at a frequency of about 1 of 180, largely irrespective of antigen or lectin stimulation. However, in four independent experiments, anti-NK-1.1 treatment was found to decrease the number of IFN-γ-secreting cells by an average of 47% (mean frequency, 1 of 336), despite the fact that anti-YAC-1 cytotoxic activity in the same cell fraction was absent or minimal (Fig. 1B). The greatest reduction (67%) was observed with unstimulated cells 1 day after infection, whereas the lowest reduction (32%) was observed with cells harvested 4 days postinfection upon overnight stimulation with heat-inactivated *C. albicans*. This suggested that in NK cell-enriched fractions, other cells in addition to NK-1.1-bearing lymphocytes might be producing IFN-γ.

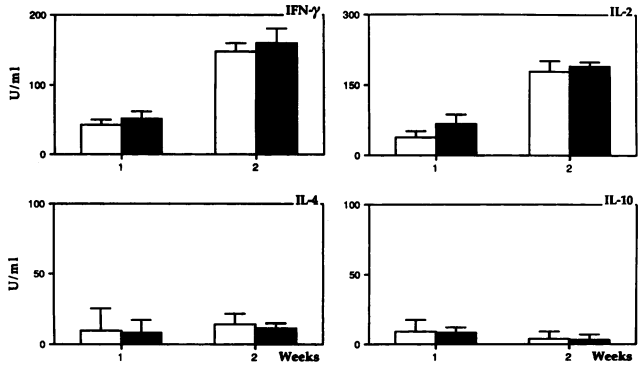

FIG. 4. Th1 and Th2 cytokine production in *C. albicans*-infected mice receiving three injections of PK136 (■) or control (□) antibodies. Mixed $CD4^+$ lymphocyte-yeast cell cultures were established at 1 and 2 weeks postinfection, and supernatants were assayed for IFN-γ, IL-2, IL-4, and IL-10 contents. Each value represents the mean ± the standard error of three to five separate experiments, each involving replicate measurements. Cytokine production in control cultures (see text) was consistently negligible.

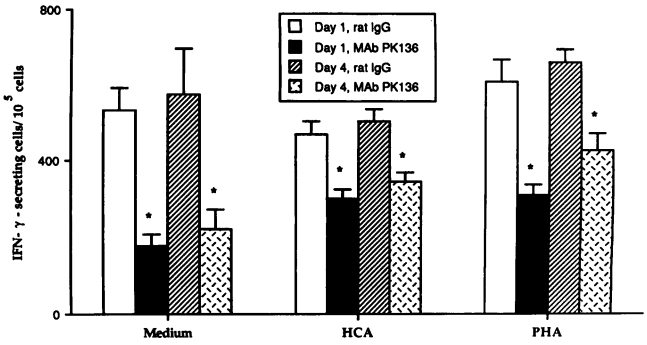

FIG. 5. Effect of in vivo administration of PK136 MAb on frequency of IFN-γ-secreting cells in Percoll-fractionated splenic populations from mice infected with *C. albicans*. Percoll fraction 2 from mice infected with the yeast for 1 or 4 days and treated with control or PK136 antibodies was assayed for frequency of cells with the potential for IFN-γ secretion under basal conditions (medium) or in response to heat-inactivated *C. albicans* (HCA) or PHA. Data are the means ± the standard errors for four experiments. The asterisk indicates that P was <0.005 (medium, day 1; PHA, day 1), <0.01 (HCA, day 4; PHA, day 4), or <0.05 (medium, day 4; HCA, day 1) for PK136 MAb versus control antibody (Student's t test).

DISCUSSION

The in vivo relevance of NK cells in the mouse resistance to inoculation candidiasis has been studied mostly by examining the course of infection in NK-deficient beige mice versus control heterozygous littermates (1, 3, 5). However, since the defect in beige mice affects the lytic capacity of NK cells but the frequency of target-binding cells remains unaltered (and so, presumably, nonlytic properties), beige mice may not be an ideal model to investigate all of the biological properties of NK cells, particularly those related to the production of cytokines (reviewed in reference 15). After the demonstration that inoculation of anti-asialo GM1 antiserum almost completely abolishes NK cell activity, this treatment has recently been employed to define the role of NK cells in the susceptibility of severe combined immunodeficient mice to systemic candidiasis (13). Because of the availability of a highly specific anti-NK cell MAb recognizing most NK cells in C57BL/6 mice (14), another NK cell-deficient mouse model has been developed by inoculating the PK136 anti-NK-1.1 MAb into C57BL/6 mice (26). We have resorted to this NK cell-deficient mouse model to investigate the role of NK cells in IFN-γ-directed development of anticandidal responses.

Consistent with the notion that C57BL/6 mice are genetically resistant to candidiasis (4, 12), mice of this strain were found to resist primary challenge with PCA-2 and develop long-lived anticandidal immunity associated with the detection of strong Th1 responses. A similar pattern of reactivity after LVS infection has been demonstrated previously for inbred parental BALB/c and hybrid (BALB/c × DBA/2)F1 (CD2F1) mice, as opposed to the development of a fatal disease and the predominance of Th2 responses in genetically susceptible DBA/2 mice (22, 24). Interestingly, CD2F1 mice also develop nonprotective Th2 responses if subjected to primary challenge with virulent CA-6 cells (23) or if treated with anti-IFN-γ MAb at the time of LVS infection (20). Therefore, *Candida*-specific Th1 cells are unable to differentiate from Th precursors in the absence of IFN-γ, and IFN-γ production by a non-$CD4^+$ cell must be considered a possible factor contributing to early differentiation

towards a predominantly Th1 phenotype. This is consistent with recent data from our laboratory that splenic $CD4^+$ cells from PCA-2-infected mice express high levels of IFN-γ transcripts at 3 days of infection, yet these cells do not account for all of the initial IFN-γ production, which is also clearly demonstrable in mice depleted of $CD4^+$ and $CD8^+$ T cells by serotherapy (21).

The ability of NK cells to respond to macrophage-derived factors such as IFN-α and IL-12 and hence release IFN-γ, and considering the finding that *C. albicans* is known to activate NK cells (9, 10, 29), makes NK lymphocytes good candidates for a determining role in $CD4^+$ subset differentiation (19). However, our present findings argue against this possibility. In fact, mice depleted of NK-1.1^+ cells at the time of LVS infection displayed no obvious changes in the qualitative or quantitative expression of their T-cell responses. This was revealed by the outcome of primary challenge, resistance to reinfection, development of yeast-specific DTH and antibody responses, and production in vitro of IFN-γ, IL-2, IL-4, and IL-10 by $CD4^+$ cells. Although NK-1.1^+ cells may not account for all NK cells in certain strains of mice (15), we would consider it unlikely that NK cells other than NK-1.1-bearing lymphocytes or those surviving serologic depletion might be responsible for the observed effect because lysis of YAC-1 tumor targets, a hallmark of murine NK cells (18), was minimal or absent in PK136-treated mice. It has been shown by others that PK136 anti-NK-1.1 MAb detects the majority of NK cells in C57BL/6 mice and, upon in vivo treatment, totally abolishes their splenic NK cell activity (26).

By quantitative analysis of IFN-γ-producing cells in vitro, we found that Percoll fraction 2 of nylon wool-nonadherent splenic populations from infected mice displayed a frequency of IFN-γ-producing cells of approximately 1 of 180. However, anti-NK-1.1 MAb treatment, which reduced the presence of $5E6^+$ cells in that fraction to 3% ± 2% (day 1) and 1% ± 2% (day 4; Fig. 1A) and abolished lysis of YAC-1 targets by day 4 (Fig. 1B), would reduce the frequency of IFN-γ-producing cells by only 32 to 67%, depending on the assay conditions. Therefore, although NK cells were activated to produce IFN-γ in mice infected with PCA-2, it is likely that only a portion of the early splenic production of IFN-γ in vitro could be attributed to NK cells, even in an NK cell-rich fraction. It is interesting to note that Percoll fraction 2 from mice infected with PCA-2 for 1 to 4 days contained as much as 50% $CD3^+$ cells, a percentage that rose to 70% in PK136-treated mice (data not shown). A detailed characterization of these T cells, most of which were $CD4^-$ $CD8^-$, will be the subject of a subsequent publication.

Although the present article deals with the effects of anti-NK.1.1^+ treatment, a similar study using anti-asialo GM1 antiserum as a means to deplete NK cells has been conducted in parallel. Despite the fact that its specificity for NK cells is questionable, anti-asialo GM1 antiserum is indeed a strong inhibitor of NK cell activity (reviewed in reference 15). By using a single intraperitoneal injection of 400 μg of polyclonal rabbit anti-asialo GM1 per mouse administered 24 h before PCA-2 infection, we obtained evidence that the NK cell deficiency caused by this treatment was not associated with impaired anticandidal resistance, changes in CD4 subset expression, or ablation of IFN-γ-producing cells in Percoll-fractionated splenic populations from infected mice (data not shown).

In a recent work by Varkila et al. (28), C.B-17 *scid* mice, which are very susceptible to infection with *Leishmania major*, were reconstituted with low numbers of T cells from nonhealer BALB/c mice, which led to a Th1-associated healing response dependent on the host's ability to produce IFN-γ. Interestingly, depletion of NK cells from infected, reconstituted *scid* mice for 3 to 4 days with a single injection of anti-asialo GM1 antiserum did not affect the development of a Th1 response. This lack of effect was taken by the authors to indicate either that NK cells did not contribute to IFN-γ production in vivo or that NK cells were not depleted for a long enough period of time.

In conclusion, the data of the present article confirm previous observations that NK cells do not play an important role in resistance to candidiasis in mice (1, 2, 13, 30) and are consistent with previous results on the lack of effect of NK cell deficiency on acquired anticandidal resistance as observed in beige mice (5). In particular, our data demonstrate that NK cells, although activated by *C. albicans* infection, are not absolutely required for the production of IFN-γ that is essential for development of Th1 responses in vaccinated mice. The early events in natural anticandidal response and the cellular sources of cytokines that will determine the phenotype of the subsequent specific response remain to be clarified.

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